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Original Article

Gender differences in nitric oxide and antioxidant response to physical stress in tissues of trained mice after hyperbaric oxygen preconditioning

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Abstract

Introduction: The aim of this study was to evaluate gender differences in the oxidant/antioxidant response in different tissues of trained mice during physical stress with the hyperbaric oxygen preconditioning (HBOP) beforehand.

Methods: Trained mice of both genders treated by HBOP were divided into three groups including basal, fasting and prolonged exercise. Parameters of oxidant/antioxidant state including nitric oxide (NO) were measured in blood and tissues. Gender differences in the effects of HBOP were analyzed in the basal levels, fasting and in the net response to exercise.

Results: HBOP diminished the elevated basal levels of lipoperoxidation, NO, antioxidant enzymes and glutathione in liver of females only, compared to untreated group. A similar decrease in the basal levels of NO and enzymes was observed in other tissues of females as well. A strong decrease of basal level of glutathione in liver coincided to its increase in muscle, brain, small intestine and adipose tissue. Therefore, females after HBOP started prolonged exercise with a lower basal level of oxidative stress and antioxidant defense in liver but increased basal levels of glutathione in most tissues. Consequently, during exercise, females showed a strong net response in the liver and stability in muscle tissue, while in males the contrary response was found.

Conclusion: Only in the tissues of females did HBOP drastically affect the basal levels of the oxidant/antioxidant status. Although performance decreased in both genders, it was better in females, likely related to the redistribution of glutathione from the liver to other tissues after HBOP.

Keywords:
Gender difference;
Oxidative stress;
Antioxidant;
Nitric oxide;
Hyperbaric oxygen

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Introduction

The mechanisms of the effect of hyperbaric oxygen preconditioning (HBOP) have yet to be elucidated. It is possible that negative effects can be induced by an HBO session by the oxidative stress that may result from a drastic increase in the concentration of oxygen in tissues, the latter have been detected by the increased concentration of peroxidation products in

the blood. There are contradictory reports in this sense, ranging from an absence of changes (Muth et al., 2004) to an increase in this parameter (Bader et al., 2006). It has been demonstrated that HBOP causes an increase in reactive oxygen species (ROS) and the antioxidant response in different tissues of rodents (Matsunami et al., 2011). But there are various studies reporting that HBOP provides a protective effect against oxidative stress caused by different factors in animal models (Kahraman et al., 2007; Cheng et al., 2011; Fuller et al., 2013; Guevara-Balcazar et al., 2015). Several studies have explored the possibility that oxidative stress and the antioxidant response are important in this protective mechanism of hyperbaric oxygenation (Thom, 2009; Castillo-Hernandez et al., 2015).

There is limited information about a gender difference in the oxidant/antioxidant response of trained rodents after HBOP. According to a previous study by our group (Kormanovski et al., 2019), among untreated mice (those without HBOP), only females showed a redistribution of glutathione (GSH) from the liver to other tissues during prolonged exercise. This redistribution coincided with elevated basal levels of nitric oxide (NO) in the majority of these tissues. There are controversial data on the effects of NO on GSH synthesis, from increase in cell-culture studies (Kuo et al., 1996) to decrease *in vivo* studies (Minamiyama et al., 1996; Payabvash et al., 2006). GSH is an abundant endogenous antioxidant synthesized in great quantities in the liver. It can be mobilized through the blood flow to tissues in the event of an imbalance between the production of ROS and the antioxidant defense. Extracellular GSH is not able to pass through cell membranes. However, after being enzymatically divided into its constituent aminoacids, the latter are indeed able to cross this barrier. Once inside the cell, these amino acids are available for the resynthesis of GSH. There is limited information about HBOP effects in NO metabolism; the increase of pulmonary (Buras et al., 2000) or cardiac levels of NO (Cabigas et al., 2006) and a vasodilator effect, increasing oxygen delivery to tissue (Buerk, 2007).

The aim of the present study was to explore, in different tissues of trained mice, the gender difference in the oxidant/antioxidant response to additional oxidative challenge of HBOP and the change in such response during the physical stress provoked by prolonged swimming. The present results are from a study that was carried out simultaneously and in the same animal model as the previously reported findings (Kormanovski et al., 2019) based on mice treated and untreated with HBOP.

Materials and methods

Animals and training protocol

All procedures and handling of the animals were in accordance with Mexican Federal Regulations for Animal Experimentation and Care (NOM-062-ZOO-1999, Ministry of Agriculture, Mexico City, Mexico). This study was approved by the Institutional Laboratory Use and Care Committee (CICUAL) of the Escuela Superior de Medicina. The professional training in swimming of humans also begins in age 7-8 years that was reflected in the age of mice in this animal model. The exclusion of some mice during the study lowered the number of animals per group (7 vs the 8 that had been contemplated).

Two-month-old male (n=21) and female (n=21) Balb/C mice were maintained in transparent plastic cages at 20-25°C on a 12h light/dark cycle with food and water available *ad libitum*. All animals were subjected to training of non-forced swimming (flotation) with a 14-week training program. The training was begun with a 2-week adaption program with swimming sessions 3 times per week. The time of exercise was gradually increased from 10 to 60min per session. Afterwards, the mice continued to train 3 times a week (60min per session) for another 12 weeks. Compared to other rodent studies involving swimming training, we consider that the present protocol represents moderate exercise training (Ilhan et al., 2004, Pepe et al., 2011). Water temperature was 32±2°C. Swimming sessions were carried out in transparent tanks divided into (25×25cm), one animal per cell. After each exercise session, mice were dried and returned to their cages. All sessions were conducted between 11am and 1pm to avoid variations due to the circadian cycle. The pilot study demonstrated that females after HBOP diminished their performance during exhaustive swimming from 297±22min to 266±18min (P<0.05) and males from 252±16min to 235±19min (P>0.05), maintaining higher performance of females (P<0.05), compared to males.

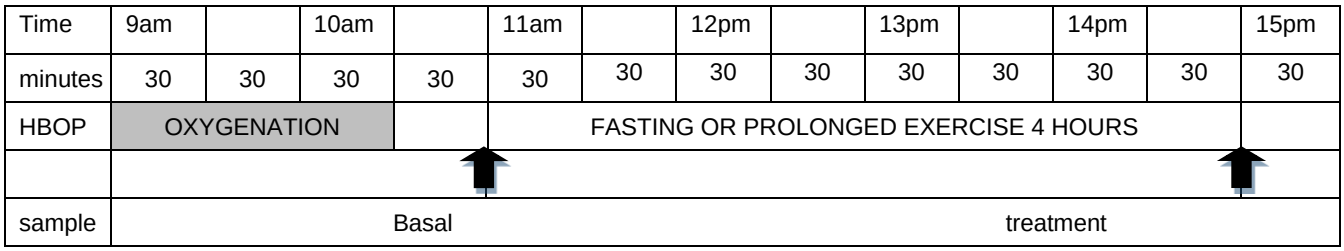

Fig.1. The time course on the experimental day. Arrows: location of basal or post-treatment sample receive.

Study design

Trained animals of the same gender were exposure to HBOP. Each gender was divided into three groups (n=7): basal, fasting and prolonged swimming exercise. The fasting and prolonged swimming both lasted 4 hours. This time was chosen for prolonged exercise because the time of exhaustive swimming was between 4.3 and 5 hours in this animal model. The time course on the experimental day was presented in following Figure 1. The basal measurement was made by sacrificing the mice 30min after the HBOP (11am). Based on previous experiments (data not shown), it was found that this HBOP led to the maximum response (30 minutes after the session) of the parameters of the oxidant/antioxidant state in different tissues in this animal model.

The fasting subgroup was included for the following two reasons: 1) the effects of HBOP changed during the 4 hours after oxygenation (Ay et al., 2007) and 2) during this time the animals were obviously fasting, although we supposed that the fast was short enough not to affect the oxidant/antioxidant state in tissues. The values measured after fasting reflect the sum of both effects, thus permitting us to eliminate the effects by comparing post-exercise with post-fasting levels (net effect of exercise). The HBOP, from 9am to 10:30am, was carried out in a hyperbaric chamber for small animals with an oxygen pressure of 2 ATA (15min pressurization, 60min exposure and 15min depressurization). Animals were returned to their cages with food and water available during 30 minutes. Both the fast and the prolonged swimming exercise in the HBOP groups began at 11am (30 minutes after the HBOP).

Tissue preparation and processing

Animals were sacrificed immediately after the respective procedure by diethyl ether anesthesia, which affects oxidant/antioxidant parameters to a lesser extent compared to other procedures. Liver, skeletal muscle from the hind leg (*vastus lateralis*), heart, brain, kidney, visceral adipose tissue and intestine (lamina propria and mucosa) were extracted and stored at -80°C to await analysis. Samples were obtained by placing a tissue in 30mmol cold phosphate buffer solution (pH 7.0) and adding 0.1% of Triton 100 (1mg of tissue per 10µl of buffer). Tissues were homogenized and centrifuged at 10,000rpm for 15min and supernatants were stored at -80°C for no more than two weeks before being analyzed. Plasma was separated from blood.

The Cayman Chemistry chemical assay kit was employed for measurement of total proteins (TP, No.704002), NO (nitrates/nitrites, No.780001) (Nims et al., 1995), total reduced glutathione (GSH) and oxidized GSH (GSSG, No.703002) (Baker et al., 1990) and the activity of catalase (CAT, No.707002) in homogenates of tissues. The Randox chemical assay kits were adapted in order to measure total antioxidant status (TAS, NX2332) (Miller et al., 1993) as well as the activity of total superoxide dismutase (SOD, No.SD125) (Arthur and Boyne, 1985) and total glutathione peroxidase (GPx, RS504) (Prohaska et al., 1977) in homogenates of tissues. Products of lipoperoxidation (thiobarbituric acid reactive substances, TBARS) were also measured (Hicks and Medina-Navarro, 1995). The values of TBARS, NO, TAS, GSH and GSSG were expressed as nmol/mg of TP and enzymes in U/mg of TP for SOD, CAT and GPx. The degree of GSH oxidation was calculated: GSSG/2GSHx100. The corticosterone level in both genders (Enzo Life Sciences, No.ADI-901-097) and estradiol level (Assay Designs, No.0460818) in females was determined in plasma by the enzyme-linked immunosorbent assay (ELISA). TAS, NO (nmol/ml), lactate (mmol/l, Randox, LC2389) were also measured in plasma. Enzymes activity and hemoglobin (HB) were also measured in whole blood

and were expressed as U/g HB.

Statistical analyses

After collection of data, descriptive statistical analysis was performed on SPSS and data are presented as the mean±SD. Statistical evaluation of parameters was performed using analysis of variances (ANOVA) followed by Tukey post hoc test. A *P* value <0.05 was considered significant. Also we analyzed the Pearson bivariate correlation between the different basal parameters measured in the all tissues in both genders and *P* value <0.05 was considered significant.

Results

HBOP affected basal and fasting levels of measured parameters in tissues of mice and consequently the net response during prolonged exercise. Compared the basal and fasting levels after HBOP of this article to the same levels of mice without treatment presented in (Kormanovski et al., 2019), we analyzed the direction of significant changes in basal levels (Table 1) and post-fasting levels (Table 2).

In males, the response of basal levels to HBOP (Table 1) was minimal and mainly found as a significant increase in NO, TAS, GSH and GSSG%. In females, the response was strong and consisted in a decrease in the aforementioned elevated (compared to males) basal parameters of non-treated mice, making them the same as or lower than those found in their male counterparts. In the majority of tissues of both genders, the most affected basal parameters were NO and GPx. Liver and muscle of females suffer highest changes in all basal parameters after HBOP (principally a decrease), while males was not showed significant changes in this organs, except an increase in NO in muscle. It is important that a decrease in basal level of GSH in liver of females was coincided to an increase in muscle, brain, intestinal mucosa and adipose tissue. Interesting there was a decrement of all parameters of oxidant/antioxidant status in liver of females, including (TBARS and NO). ce. Finally higher basal levels in majority tissues of females in non-treated trained mice drastically decreased by HBOP that was not observed in males.

Compared fasting levels after HBOP to fasting levels of non-treated mice, the changes (principally a decrease of NO, GPx and GSH) was observed also in females (Table 2). There was a decrease in GSH level in liver, brain and adipose tissue of females during fasting without change in its grade of oxidation, accompanied by an increase in small intestine. Basal, post-fasting and post-exercise levels after HBOP in liver, skeletal muscle, heart and brain of both genders were presented in Table 3.

Liver

When comparing males and females after HBOP, females showed a significantly lower basal level of all parameters (*P*<0.01, except GPx) but higher basal level of the ratio GPx/GSH (*P*<0.01), confirmed protective effects of HBOP against oxidative stress (TBARS and NO) that coincided to a decreased level of antioxidant defense in liver of females. The same gender differences were maintained after fasting including lower level of GPx (*P*<0.01) and higher level of GSSG% (*P*<0.05). Compared to basal levels, fasting was not affected measured parameters in males, while females showed a decrease in SOD, GPx and GSH (*P*<0.01) that confirmed decrease in antioxidant defense in liver of females including oxidation of GSH during relatively short fasting.

In the liver gender differences were displayed in lower post-exercise levels of TAS (*P*<0.05), SOD, CAT (*P*<0.01) and GSH (*P*<0.01) (without changes in GSSG%) in females and higher level of ratio GPx/GSH (*P*<0.01). When comparing exercise levels to fasting levels (net response during exercise), females showed an increase in oxidative stress (TBARS and NO, *P*<0.01) and levels of SOD and GPx (*P*<0.01) while males was not showed significant response, except moderate increase in lipoperoxidation. This response reflects the greater sensitivity of the liver tissue in females than males in respect to the parameters of the oxidant/antioxidant status after HBOP.

Muscle

When comparing males and female after HBOP, the latter showed greater basal level of TBARS, GPx and the ratio GPx/GSH (*P*<0.01) and lower basal levels of NO, TAS and GSH (*P*<0.01), confirmed the elevated basal level of lipoperoxidation and decreased levels of TAS, GSH and NO. Only muscle of females showed higher level of GPx after fasting (*P*<0.01), compared to males. Compared fasting to basal

Table 1: Basal levels of oxidant/antioxidant state parameters. Direction of significant changes in basal levels in different tissues of both genders, induced by hyperbaric oxygen preconditioning.

HBOP basal levels	TBARS		NO		TAS		SOD		CAT		GPX		GSH		GSSG%	
	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f
liver		D		D		D		D		D		D		D		
muscle		I	I	D		D				D		D		I		I
brain														I	D	D
heart			I												I	
kidney		D	I	D				D		D						
lamina propria				D		D					D	D			I	I
mucosa				D	I							D	I	I		I
adipose tissue		I			I									I		
f: female, m: male, D: decrease, I: increase, empty: no significant change.																

Table 2: Fasting levels oxidant/antioxidant state parameters. Direction of significant changes of measured parameters during fasting in tissues of both genders, after hyperbaric oxygen preconditioning.

HBOP fasting	TBARS		NO		TAS		SOD		CAT		GPX		GSH		GSSG%	
	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f
liver								D				D		D		
muscle	I		D	I								D	D			
brain						D				D		I		D	I	
heart											I	D				
kidney		I	D			I		I		I			I			
lamina propria														I	D	I
mucosa				D									D	I		
adipose tissue				D										Di		
f: female, m: male: D: decrease, I: increase, empty: no significant change.																

levels, males were presented a decrease of NO (P<0.01), while females contrarily an increase in NO, ratio GPx/GSH and a decrease in GPx (P<0.01). The drastic increase in NO could be beneficial for muscles of females during fasting due to its vasodilator effect. Lower post-exercise levels showed females in all parameters (except GPx and GSSG%) compared to males, confirmed a lower level as oxidative stress as the antioxidant defense in females after exercise of HBO treated mice. The net response during exercise included in males an increase in NO, CAT and GSH (P<0.01) and tendency to an increase in other enzymes, while females showed only decrease in GSH and increase in ratio GPx/GSH (P<0.01). The net response during exercise in males was strong that was not observed in females.

Heart

The heart of females showed a greater basal level of NO, GPx and GSH, and lower GSSG% level (P<0.01) compared to males. The post-fasting levels showed higher NO level (P<0.01) in females only compared to males. Compared to basal levels, males showed an

Table 3: Gender differences in liver, muscle, heart and brain. The levels of measured parameters (mean±SD) are indicated for basal, post-fasting (4h) and post-exercise (4h) in tissues of both genders after hyperbaric oxygen preconditioning.

	BASAL		FASTING		EXERCISE	
	male	female	male	female	male	female
LIVER						
NO	3.4 ± 0.7	1.4 ± 0.5**	4.6 ± 1.2	1.8 ± 0.8**	4.9 ± 0.9	4.1 ± 1.5$^{\#\#}$
TBARS	1.8 ± 0.2	0.98 ± 0.3**	1.9 ± 0.4	0.9 ± 0.2**	2.4 ± 0.2$^{\#}$	2.1 ± 0.5$^{\#\#}$
TAS	3861 ± 431	1772 ± 283**	3587 ± 497	1512 ± 195**	3866 ± 568	1941 ± 1000*
SOD	108 ± 29	44.4 ± 6.2**	81 ± 26	27 ± 2**$^{\&\&}$	93 ± 14	49 ± 21**$^{\#\#}$
CAT	1074 ± 135	500 ± 66**	1106 ± 195	426 ± 85**	1356 ± 176	424 ± 136**
GPx	1092 ± 255	1336 ± 319	1467 ± 309	618 ± 63**$^{\&\&}$	1706 ± 227	1318 ± 505$^{\#\#}$
GSH	211 ± 36	103 ± 17**	190 ± 31	71 ± 8**$^{\&\&}$	199 ± 39	91 ± 18**
GPx/GSH	5.2 ± 2.1	13.0 ± 4.2**	7.5 ± 3.7	8.7 ± 4.1	8.6 ± 3.9	14.5 ± 3.7**
GSSG%	0.66 ± 0.34	0.44 ± 0.20	0.47 ± 0.15	1.18 ± 0.48*$^{\&\&}$	0.57 ± 0.15	0.74 ± 0.50
MUSCLE						
NO	7.0 ± 1.4	0.84 ± 0.8**	2.0 ± 0.5$^{\&\&}$	4.0 ± 1.3$^{\&\&}$	6.1 ± 0.8$^{\#\#}$	3.4 ± 1.4**
TBARS	3.5 ± 1.0	5.7 ± 0.8**	5.8 ± 1.5	4.1 ± 1.1	5.5 ± 2.4	3.8 ± 1.3*
TAS	63.0 ± 18.2	34 ± 9**	54 ± 18	31 ± 21	75 ± 20	35 ± 17**
SOD	25.6 ± 9.4	22.7 ± 4.9	20 ± 9	13.2 ± 5.5	35 ± 13	18.3 ± 6.6*
CAT	10.2 ± 2.6	7.8 ± 4.1	11.8 ± 2.0	10.5 ± 1.9	17.8 ± 2.2$^{\#\#}$	8.6 ± 2.9**
GPx	78 ± 28	290 ± 51**	58 ± 7	105 ± 15**$^{\&\&}$	97 ± 41	132 ± 40
GSH	7.6 ± 2.8	3.0 ± 0.5**	4.0 ± 1.8	3.6 ± 0.2	7.1 ± 2.0$^{\#\#}$	1.7 ± 0.5**$^{\#\#}$
GPx/GSH	10 ± 4.5	97 ± 29**	15 ± 5.0	29 ± 11.4$^{\&\&}$	14 ± 4.5	78 ± 29**$^{\#\#}$
GSSG%	4.6 ± 1.9	3.7 ± 1.0	7.4 ± 3.3	3.3 ± 2.3	3.6 ± 2.0	3.3 ± 2.2
HEART						
NO	2.4 ± 1.1	6.4 ± 1.8**	1.7 ± 0.5	6.8 ± 1.8**	3.6 ± 1.5	5.7 ± 0.3*
TBARS	3.7 ± 1.2	4.5 ± 1.5	3.1 ± 0.7	4.2 ± 1.0	2.6 ± 1.0	6.1 ± 1.5**
TAS	101 ± 35	131 ± 20	118 ± 42	112 ± 12	107 ± 22	144 ± 37
SOD	15.9 ± 7.4	23.2 ± 8.8	12.8 ± 7.5	14.8 ± 5.4	12.4 ± 5.6	23 ± 10
CAT	48.4 ± 16.5	63.6 ± 9.4	53 ± 9	64 ± 14	59 ± 16	59 ± 10
GPx	171 ± 46	584 ± 189**	284 ± 56$^{\&\&}$	285 ± 71$^{\&\&}$	234 ± 32	476 ± 195**$^{\#}$
GSH	0.59 ± 0.26	1.1 ± 0.25**	0.57 ± 0.12	0.71 ± 0.11$^{\&}$	1.0 ± 0.4	1.3 ± 0.3$^{\#}$
GPx/GSH	285 ± 133	531 ± 176	473 ± 185	407 ± 136	234 ± 98	366 ±144
GSSG%	3.9 ± 0.9	1.3 ± 0.3**	1.8 ± 0.8$^{\&\&}$	1.0 ± 2.1	2.9 ± 1.1$^{\#}$	1.2 ± 0.8
BRAIN						
NO	4.2 ± 1.4	3.7 ± 1.9	4.3 ± 2.7	3.2 ± 0.8	6.1 ± 2.7	3.9 ± 1.6
TBARS	7.1 ± 2.4	8.1 ± 1.7	6.8 ± 3.8	6.9 ± 1.3	8.7 ± 1.7	7.2 ± 1.6
TAS	131 ± 25	151 ± 58	94 ± 41	87 ± 17	152 ± 34	95 ± 25**
SOD	93 ± 49	81 ± 32	78 ± 59	37 ± 19	82 ± 20	72 ± 19 $^{\#}$
CAT	22 ± 8	36 ± 18	21 ± 9	19.3 ± 5.9	19.2 ± 7.7	23 ± 8
GPx	147 ± 53	274 ± 78*	97 ± 44	561 ± 185**$^{\&\&}$	158 ± 54	354 ± 186
GSH	2.3 ± 1.0	1.4 ± 0.5	1.8 ± 0.8	0.74 ± 0.19**$^{\&\&}$	1.5 ± 0.2	1.1 ± 0.2**$^{\#}$
GPx/GSH	64 ± 39	196 ± 133**	54 ± 24	801 ± 287**$^{\&\&}$	105 ± 39	322 ± 167**
GSSG%	2.8 ± 0.9	0.64 ± 0.38**	4.4 ± 1.5	0.60 ± 0.21**	2.2 ± 0.84$^{\#}$	0.67 ± 0.34**

$^{*}P<0.05$, $^{**}P<0.01$: gender differences. $^{\&}P<0.05$, $^{\&\&}P<0.01$ compared fasting to basal levels. $^{\#}P<0.05$, $^{\#\#}P<0.01$ compared exercise to fasting levels of the same gender. TBARS: thiobarbituric acid reactive substances, NO: nitric oxide, TAS: total antioxidant status, GSH: total reduced glutathione, GPx: glutathione peroxidase, SOD: superoxide dismutase, CAT: catalase, GSSG: oxidized GSH. Units: NO, TAS, GSH and TBARS (nmol/mg proteins), GPx, SOD and CAT (U/mg proteins), GPx/GSH (U/nmol×1000) and GSSG% (GSSG/2GSH×100).

Table 4: Gender differences in small intestine, kidney and adipose tissue. The levels of measured parameters (mean±SD) are indicated for basal, post-fasting (4h) and post-exercise (4h) in tissues of both genders after hyperbaric oxygen preconditioning.

	BASAL		FASTING		EXERCISE	
	male	female	male	female	male	female
LAMINA PROPRIA						
NO	3.2 ± 0.5	5.2 ± 1.7*	4.6 ± 1.3	3.7 ± 1.8	7.0 ± 0.8$^{\#\#}$	8.2 ± 2.2$^{\#\#}$
TBARS	2.1 ± 0.5	2.8 ± 0.5	2.7 ± 0.5	2.3 ± 0.4	3.0 ± 0.5	2.7 ± 0.5
TAS	245 ± 48	241 ± 72	320 ± 103	180 ± 24**	378 ± 76	369 ± 67$^{\#\#}$
SOD	62 ± 19.1	83 ± 13	80 ± 22	54 ± 12$^{\&}$	96 ± 22	93 ± 24$^{\#\#}$
CAT	16.0 ± 3.4	25 ± 4.3*	21 ± 6.7	14 ± 1.4$^{\&}$	23 ± 4.2	24 ± 5.3$^{\#\#}$
GPx	194 ± 63	313 ± 72*	256 ± 95	231 ± 45$^{\&}$	410 ± 91$^{\#\#}$	290 ± 42**$^{\#\#}$
GSH	0.58 ± 0.15	1.10 ± 0.34**	0.64 ± 0.19	2.0 ± 0.5**$^{\&\&}$	0.72 ± 0.15	2.5 ± 1.1**
GPx/GSH	334 ± 112	285 ± 97	400 ± 165	116 ± 56**$^{\&\&}$	569 ± 222	119 ± 59**
GSSG%	2.1 ± 0.3	3.1 ± 0.9	0.70 ± 0.32$^{\&}$	6.0 ± 3.6**$^{\&\&}$	0.88 ± 0.13	21 ± 9.2**$^{\#\#}$
MUCOSA						
NO	5.9 ± 0.9	8.5 ± 2.1*	6.1 ± 1.7	4.5 ± 2.3$^{\&}$	4.6 ± 0.8	3.9 ± 1.9
TBARS	2.8 ± 0.5	2.9 ± 0.9	2.4 ± 1.1	3.2 ± 1.2	2.0 ± 0.4	2.4 ± 0.7
TAS	368 ± 47	245 ± 74*	373 ± 134	270 ± 92	355 ± 44	159 ± 40**$^{\#}$
SOD	100 ± 32	68 ± 23	90 ± 35	79 ± 44	92 ± 17	50 ± 22**
CAT	81 ± 25	57 ± 28	77 ± 32	30 ± 16	72 ± 22	20 ± 7**
GPx	272 ± 39	184 ± 55*	261 ± 114	263 ± 105	288 ± 121	218 ± 50
GSH	0.91 ± 0.14	3.4 ± 1.8*	0.55 ± 0.23	6.0 ± 4.5**$^{\&\&}$	0.69 ± 0.13	2.9 ± 1.7**$^{\#\#}$
GPx/GSH	299 ± 154	54 ± 18**	475 ± 198	44 ± 13**	417 ± 175	75 ± 29**
GSSG%	0.44 ± 0.50	4.3 ± 0.9**	0.18 ± 0.11	4.4 ± 3.4**	0.08 ± 0.03	2.4 ± 0.7**$^{\#\#}$
KIDNEY						
NO	2.5 ± 0.5	2.6 ± 1.9	1.5 ± 0.3$^{\&\&}$	2.6 ± 0.6**	2.4 ± 0.7	4.3 ± 1.5*$^{\#}$
TBARS	1.9 ± 0.4	1.2 ± 0.4	1.9 ± 0.4	4.0 ± 1.2**$^{\&\&}$	1.7 ± 0.2	3.3 ± 1.5*
TAS	2146 ± 288	1343 ± 209**	2303 ± 259	3532 ± 620**$^{\&\&}$	2466 ± 266	3835 ± 982**
SOD	14.1 ± 3.4	4.5 ± 1.7**	13.8 ± 3.6	11.6 ± 2.1$^{\&\&}$	15.2 ± 2.5	10.1 ± 4.5
CAT	277 ± 46	111 ± 31*	359 ± 225	312 ± 62$^{\&\&}$	306 ± 35	300 ± 134
GPx	282 ± 117	542 ± 171*	383 ± 170	312 ± 178	162 ± 78$^{\#}$	300 ± 209
GSH	0.42 ± 0.15	0.27 ± 0.16	0.80 ± 0.51	0.51 ± 0.25	0.47 ± 0.17	1.21 ± 0.5**$^{\#\#}$
GPx/GSH	671 ± 209	2007 ± 812**	480 ± 202	612 ± 285	345 ± 199	248 ± 143
GSSG%	4.6 ± 1.8	2.6 ± 1.9	4.1 ± 2.6	3.7 ± 1.2	9.9 ± 2.1$^{\#\#}$	2.8 ± 2.3**
TEJIDO ADIPOSO						
NO	1.5 ± 0.4	2.5 ± 1.2	1.7 ± 0.6	1.2 ± 0.7	1.8 ± 0.5	1.6 ± 0.8
TBARS	1.7 ± 0.2	1.5 ± 0.4	1.6 ± 0.2	1.2 ± 0.2**	1.2 ± 0.5	1.1 ± 0.3
TAS	37 ± 5	43 ± 8	25 ± 11	39 ± 14	22 ± 3	36 ± 7**
SOD	0.24 ± 0.06	0.29 ± 0.06	0.29 ± 0.13	0.37 ± 0.12	0.14 ± 0.03	0.29 ± 0.08**
CAT	9.0 ± 10.2	8.5 ± 2.5	9.2 ± 2.4	10.0 ± 2.0	8.9 ± 1.9	5.9 ± 1.3*$^{\#\#}$
GPx	33 ± 9	42 ± 10	32 ± 9	32 ± 8	119 ± 10$^{\#\#}$	25 ± 7**
GSH	0.36 ± 0.12	1.7 ± 0.19**	0.23 ± 0.10	0.86 ± 0.27**$^{\&\&}$	0.29 ± 0.08	0.90 ± 0.26**
GPx/GSH	92 ± 43	25 ± 9**	139 ± 54	37 ± 18**	410 ± 211$^{\#\#}$	28 ± 13**
GSSG%	2.2 ± 2.5	11.4 ± 2.1**	1.9 ± 0.7	13.5 ± 5.1**	7.4 ± 4.4$^{\#}$	27.6 ± 14.4**

$^{*}P<0.05$, $^{**}P<0.01$: gender differences. $^{\&}P<0.05$, $^{\&\&}P<0.01$ compared fasting to basal levels. $^{\#}P<0.05$, $^{\#\#}P<0.01$ compared exercise to fasting levels of the same gender. TBARS: thiobarbituric acid reactive substances, NO: nitric oxide, TAS: total antioxidant status, GSH: total reduced glutathione, GPx: glutathione peroxidase, SOD: superoxide dismutase, CAT: catalase, GSSG: oxidized GSH. Units: NO, TAS, GSH and TBARS (nmol/mg proteins), GPx, SOD and CAT (U/mg proteins), GPx/GSH (U/nmol×1000) and GSSG% (GSSG/2GSH×100).

Table 5: Net response during prolonged exercise. Direction of significant changes of measured parameters during prolonged exercise in tissues of both genders, after hyperbaric oxygen preconditioning.

HBOP exercise	TBARS		NO		TAS		SOD		CAT		GPX		GSH		GSSG%	
	m	f	m	f	m	f	m	f	m	f	m	f	m	f	m	f
liver		I		I		I						I				
muscle			I						I		D		I	D		
heart												I		I	I	
brain														I		
kidney			I	I							D			I	I	
lamina propria			I	I		I		I		I	I					I
mucosa						D								D		
adipose tissue										D	I				I	I

f: female, m: male, D: decrease, I: increase, empty: no significant change.

Table 6: Gender differences in whole blood and plasma. The levels of measured parameters (mean±SD) are indicated for basal, post-fasting (4h) and post-exercise (4h) in blood of both genders after hyperbaric oxygen preconditioning

blood	BASAL		FASTING		EXERCISE	
	male	female	male	female	male	female
TBARS	6.1 ± 1.0	8.7 ± 1.5*	7.9 ± 2.2	7.7 ± 2.7	6.0 ± 1.5	4.6 ± 1.9
NO	8.7 ± 2.1	30.0 ± 10.3**	18.7 ± 6.2$^{\&\&}$	20.3 ± 11.3$^{\&}$	17.4 ± 9.8	11.5 ± 2.0
TAS	739 ± 155	715 ± 205	951 ± 363	1033 ± 123	731 ± 130	969 ± 295
SOD [b]	3024 ± 527	2420 ± 507	2361 ± 295	2599 ± 1199	4108 ± 848$^{\#\#}$	2449 ± 811**
CAT [b]	149 ± 52	154 ± 24	116 ± 29	121 ± 62	132 ± 29	118 ± 44
GPx [b]	1149 ± 92	1798 ± 546*	1163 ± 217	1374 ± 215	1208 ± 126	1808 ± 449**
HB [b]	14.3 ± 1.2	13.9 ± 1.2	15.5 ± 0.6	13.1 ± 1.1	15.1 ± 0.5	13.6 ± 0.8
LA	5.0 ± 1.2	5.4 ± 1.5	4.6 ± 1.3	5.6 ± 1.8	2.3 ± 1.4	2.0 ± 1.3$^{\#\#}$
CST	50.9 ± 10.4	29.5 ± 10.3*	30.8 ± 13.8	28.8 ± 14.7	64.6 ± 8.2$^{\#\#}$	58.7 ± 5.5$^{\#\#}$
EST		75 ± 23		80 ± 19		77 ± 21

$^{*}P$<0.05, $^{**}P$<0.01: gender differences. $^{\&}P$<0.05, $^{\&\&}P$<0.01 compared fasting to basal levels of the same gender. $^{\#}P$<0.05, $^{\#\#}P$<0.01 compared exercise to fasting levels of the same gender. [b]: whole blood, HB: hemoglobin, LA: lactate, CST: corticosterone (ng/ml), EST: estradiol (pg/ml).

increase in GPx and decrease in GSSG% (P<0.01) during fasting, while females showed a decrease in GPx P=0.01) and GSH (P<0.05). Post-exercise levels showed higher levels of TBARS (P<0.05), NO and GPx (P<0.01) in females, compared to males. The net response to prolonged exercise also led to an increase in females of GPx and GSH (P<0.05) that was not observed in males.

Brain

After HBOP there was elevated basal levels of GPx (P<0.05), ratio GPx/GSH and a lower level of the of GSSG% (P<0.01) in brain of females versus males. Regarding fasting, gender differences existed in GPx and ratio GPx/GSH (P<0.01) (higher in females), in GSH and GSSG% (P<0.01) (lower in females). Compared to basal levels, fasting was not affected

parameters in males, while in females increased GPx and ratio GPx/GSH levels (P<0.01) and decreased GSH levels (P<0.01). Gender differences in post-exercise levels included lower levels in TAS, GSH and GSSG% and higher levels of GPx/GSH (P<0.01) in females versus males. Net response during exercise showed an increase in SOD and GSH (P<0.05) in females and GSSG% (P<0.05) in males.

Basal, fasting and post-exercise levels after HBOP in lamina propria and mucosa of small intestine, kidney and adipose tissue of both genders were presented in Table 4.

Small intestine (lamina propria and mucosa)

After HBOP was observed the higher basal level of NO, CAT, GPx (P<0.05) and GSH (P<0.01) in lamina propria of females, compared to males. Fasting also led to gender differences in TAS and GPx/GSH (P<0.01) (lower in females) as well as GSH and GSSG% (P<0.01) (higher in females). Compared to basal levels, only females showed significant changes during fasting, a decrease in all enzymes (P<0.05) and the ratio GPx/GSH (P<0.01), and strong increase in GSH and GSSG% (P<0.01). Lamina propria of females showed gender differences in post-exercise levels, lower levels of GPx and GPx/GSH (P<0.01) and higher levels in GSH and GSSG% (P<0.01). The net response during exercise showed principally changes in females that included an increase in NO and TAS (P<0.01), all enzymes (P<0.01) and GSSG% (P<0.01), while males showed an increase in NO and GPx only.

Gender differences in basal levels of mucosa intestinal after HBOP included higher level of NO, GSH (P<0.05) and GSSG% (P<0.01) and lower levels in TAS and GPx (P<0.05) in females. Fasting levels after HBOP showed higher levels in GSH and GSSG% (P<0.01) and lower ratio GPx/GSH (P<0.01) in females only. Males was not shown significant changes during fasting, while females presented a decrease in NO (P<0.05) and an increase in GSH (P<0.01). Post-exercise levels in mucosa intestinal showed lower levels in TAS, enzymes (except GPx) and GPx/GSH (P<0.01) and higher levels in GSH and GSSG% (P<0.01) in females, versus males. Compared to fasting levels, females showed a decrease in TAS (P<0.05), GSH and GSSG% (P<0.01) (net response during exercise), while males was not showed significant changes. Compared response in lamina propria and mucosa, females showed an increase of antioxidant status in lamina propria and a decrease in mucosa, while the response in small intestine of males was minimal. This gender differences coincided to higher basal and post-fasting levels of NO in females.

Kidney

Females after HBOP had a lower basal level of TAS, SOD (P<0.01) and CAT (P<0.05) in kidney, compensated by a greater basal level of GPx (P<0.05) and GPx/GSH (P<0.01). Compared to males, females showed higher levels of TBARS, NO and TAS (P<0.01) in post-fasting levels after HBOP, confirmed oxidative stress in kidney of this gender during fasting. Compared to basal levels, fasting levels in males was not changed (except a decrease in NO, P<0.05), while females presented an increase in TBARS, TAS, SOD and CAT (P<0.01). Post-exercise levels of NO, TBARS (P<0.05), TAS and GSH (P<0.01) were greater and GSSG% (P<0.01) lower in kidney of females, compared to males. The net response during exercise included in kidney of males a decrease in GPX (P<0.05) and an increase in GSSG% (P<0.01), while females showed an increase in NO (P<0.05) and GSH (P<0.01).

Adipose tissue

Gender differences in basal levels after HBOP included higher levels of GSH and GSSG% (P<0.01) in adipose tissue of females versus males. Post-fasting levels showed lower levels in TBARS and GPx/GSH (P<0.01) and greater levels in GSH and GSSG% (P<0.01) of females compared to males. Compared to basal levels, males was not presented changes during fasting in adipose tissue, while females showed a decrease in GSH level (P<0.01). Post-exercise levels expressed higher levels of TAS, SOD, GSH and GSSG% (P<0.01), and lower levels of CAT (P<0.05), GPx and GPx/GSH (P<0.01) in females versus males. Net response during exercise included in males an increase in GPx, GPx/GSH (P<0.01) and GSSG% (P<0.05), while in females a decrease of CAT (P<0.01) only.

The summary of the directions of significant net response during prolonged exercise in oxidant/antioxidant parameters in different tissues was presented in Table 5. An increase of majority of measured parameters was principal net response

after HBOP. Both genders showed significant net response of NO and GPx during exercise in majority of tissues (Table 5). Liver of females showed an increase in indicators of oxidative stress (TBARS and NO), and in TAS and GPx. This response of liver after HBOP coincided to the stability in all parameters in muscle of females that coincided to a decrease in GSH. Other tissue of females with strong response was lamina propria of small intestine.

Blood

The data on the oxidant/antioxidant parameters in whole blood and plasma of trained mice, for both genders after HBOP, are denoted in Table 6. When comparing males and females after HBOP, females showed a significantly greater level basal levels of TBARS ($P<0.05$), NO ($P<0.01$) and GPx ($P<0.05$), but lower level of CST ($P<0.05$) in plasma. There was not observed gender differences in post-fasting levels in blood after HBOP. Compared to basal levels, males showed strong increase in NO ($P<0.01$) and decrease in CST ($P<0.05$) after fasting, while females a decrease in NO ($P<0.05$). Post-exercise levels presented lower levels of SOD and higher levels of GPx ($P<0.01$) in females versus males. Net effect during exercise in blood of males showed an increase of SOD and ($P<0.01$), and females a decrease in lactate ($P<0.01$), while both genders presented an increase in concentration of CST ($P<0.01$) that corresponded to physical stress.

We analyzed the bivariate correlation coefficients between the different basal parameters measured after HBOP in both genders in the various tissues presented in Table 7, excluding liver and kidney tissue (where the majority of parameters were over 10-fold greater than in other). Multiple bivariate correlations between basal parameters measured in different tissues is an indicator that there is a close relation between some parameters in all tissues. The level of NO positively correlated with TAS ($P<0.05$) and SOD ($P<0.01$) in both genders, and also with CAT ($P<0.05$) in females, which suggests a close relation between NO and parameters of antioxidant defense. Variations in TAS in males positively correlated with the changes in all enzymes ($P<0.05$) while in females with SOD ($P<0.05$) only. This coincides with our previously reported data in human ocular tissue (Kormanovski et al., 2014) and confirms that enzymes together with nonenzymatic antioxidants contribute to value of TAS.

Discussion

Two distinct effects of HBOP sessions have been reported in relation to oxidative stress. Comparing hyperbaric oxygenation effects to basal levels, an increase in oxidative stress and the antioxidant response has been reported (Matsunami et al., 2011, Dennog et al., 1999). There are reports that an increase in cardiac levels of NO (Cabigas et al., 2006) provokes a vasodilator effect, increasing oxygen delivery to tissue (Buerk, 2007). Various studies explore the possibility that oxidative stress plays an important role in the mechanism of the effects produced by hyperbaric oxygenation (Thom, 2009; Castillo-Hernandez et al., 2015; Sun et al., 2011). On the other hand, there are several reports of a protective effect of a HBOP session against oxidative stress produced by distinct factors in different animal models such as experimental damage in rat spinal cord (Kahraman et al., 2007), cerebral ischemia-reperfusion (Cheng et al., 2011), experimental myocardial infarct (Sun et al., 2011) and UV light (tested for its effect on mouse skin and liver) (Fuller et al., 2013). In another study, HBOP diminished indicators of oxidative stress in rat thoracic aorta (Guevara-Balcazar et al., 2015). Recent study was showed that HBOP decreased oxidative stress and increased activity of antioxidant enzymes in rat lung probably by the up-regulation of heme-oxidase-1 (Feng et al., 2015).

In this study HBOP mainly affected elevated basal levels of measured parameters in non-treated females, decreasing them in different tissues. In particular, it diminished the basal level of NO in the majority of tissues, although this parameter was unchanged in the brain and rose in the heart. The latter are two very important tissues for adaptation to stress. In the present study, liver and skeletal muscle of females showed high sensitivity to HBOP, with a decrease in basal levels of indicators of oxidative stress and antioxidant defense. This sensitivity in females compared to minimal change in males (found in both tissues) could be due to several possible reasons.

Firstly, this effect coincided with a significant decrease in the basal level of estrogens in the blood of non-treated females after HBOP (from 127 to

75ng/ml) that probably reflected the relation of this parameter with oxidative stress and antioxidant defense in females. The pros and cons of this relation can be discussed (Tiidus and Enns 2009). The authors consider oxidative stress and the antioxidant response as one of the possible mechanisms of the effects of estrogens, due to their direct antioxidant capacity, and also indirectly through the regulation of NOS (Prorock et al., 2003). The decrease of basal estrogens level after HBOP was coincided to an increase in plasmatic levels of TBARS in both genders, and strong increase of plasmatic NO in females, while in liver and muscle of females this indicators decreased, confirmed as direct as indirect possible mechanism of basal estrogens antioxidant effects in this animal model.

Secondly, the sharp drop in the basal level of NO after HBOP in female mice, probably causing vasoconstriction, increases blood circulation in both of these tissues, and therefore results in lower basal levels of indicators of oxidative stress. Consequently, there is a decrease in the need for an antioxidant defense. This assumption is supported by the potent vasodilator effect of NO, the speed of the response and the similar degree of decrease (around 60%) in the parameters of female mice 30min after the HBOP.

Thirdly, the 64% increase in the basal level of plasma corticosterone in males after the HBOP found in the present study (without a gender difference in basal level of non-treated mice) shows greater stress in these animals than in females induced by HBOP. It can be expected that a greater basal level of corticosterone provoked oxidative stress in males and would lead to a greater antioxidant response, which could explain the lower degree of change in males after the HBOP. This supposition is supported by results of different studies (Zafir and Banu, 2009), confirming that glucocorticoids induce a significant increase on oxidative stress.

Fourthly, the basal level of GSH after HBOP decreased drastically in the liver of females, while increasing in the three tissues most abundant in mice (muscle, intestine and adipose tissues), and in brain, leaving the remaining tissues without any significant effect. Taking into account that the basal level of GSH is at least 50 times greater in liver than in other tissues, these data imply the redistribution of basal GSH toward other tissues after HBOP in females. That is, the decrease in the basal antioxidant defense in females caused by HBOP in various tissues was compensated by the mobilization of GSH from the liver. The basal level of the degree of oxidation of GSH in females increased in the majority of tissues and was unchanged in others, confirmed his greater participation in antioxidant defense in this gender. Meanwhile, males after HBOP showed no change in the basal level of GSH in the majority of tissues.

Females after HBOP began exercise with lower basal levels of indicators of oxidative stress and the antioxidant defense (except GPx) in the liver, kidney and muscle tissue, but higher basal levels of the antioxidant defense in other tissues, including the blood. Gender differences were multiple in relation to the net effect of prolonged exercise following HBOP, such as the increased levels of lipoperoxidation (TBARS) and parameters of the antioxidant defense (TAS, SOD, GPx and GSH) in the liver of females. The drastic decrease (57%) of basal level of GSH in liver of non-treated females after HBOP was observed, following by a decrease (30%) during fasting with possible its redistribution to other tissues, strengthening its initial antioxidant defense. This effect can be the reason of the mobilization impossibility of decreased level GSH from liver of females during prolonged exercise that was observed after HBOP in this study. This would explain the diminished velocity of depletion of antioxidant enzymes in this organ of females. But the net response of GSH during exercise after HBOP showed also a decrease of GSH in muscle and mucosa of females that coincided to its increase in heart, brain and kidney, confirmed possible redistribution of GSH from other tissues during exercise in females. Since the performance of mice in exhaustive swimming after HBOP showed significant decreased, higher in females, but remains higher, compared to males (see Methods).

Finally, additional oxidative challenge of HBOP diminished the elevated basal levels of NO and the antioxidant defense in the tissues of females. Whereas a strong pro-oxidative effect was mainly observed in the liver during prolonged exercise, in other tissues (muscle, heart, brain and spleen) the capacity to counteract oxidative stress during exercise remained high in females compared to males. There is evidence that HBOP decreased the basal antioxidant defense in various tissues of

females, while at the same time strengthening this capacity by facilitating the redistribution of basal or fasting GSH from the liver to other tissues, which allowed females to avoid the development of oxidative stress in muscle and brain tissues. The mechanism of this effect of HBOP in females needs the investigation, but it is possible that NO have important paper in this mechanism. Also, there is necessary the evaluation of the response of 3-nitrotyrosine as marker of nitrosative stress and different nitric oxide synthases in the same animal model.

Conclusion

HBOP mainly decreased basal levels of NO, oxidative stress and antioxidant defense in majority tissues of females only that coincided to a decrease in their performance. For females there was a sharp increase in the oxidant/antioxidant response during prolonged exercise in liver and relative stability in muscle, while in males the contrary response was found.

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Conflict of interest

The authors have no conflicts of interest and declare that this article has not been published.

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